IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	James S. Reid et al.		
Application No.	:	09/129,028	Confirmation No. :	3865
Filed	:	August 4, 1998		
For	:	METHODS FOR TREATING NEUROLOGICAL DEFECTS		
Group Art Unit	:	1649		
Examiner	:	Chang Yu Wang		

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Hon. Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

DECLARATION OF JAMES H. FALLON UNDER 37 C.F.R. § 1.132

Sir:

I, Dr. James H. Fallon, state the following:

1. I am the same James H. Fallon who executed a Declaration in the above-identified application on March 4, 2009 ("the Fallon I Declaration"). I understand that the Fallon I Declaration was filed in the United States Patent and Trademark Office in this application on May 5, 2009.

2. I submit this Declaration ("the Fallon II Declaration") to provide a report of two sets of experiments that were conducted in my laboratories at the University of California

(Irvine). I personally planned, supervised and directed these experiments. In fact, I personally made the hand-drawings that are attached as Exhibits A, D and E hereto contemporaneously with the experiments from which they resulted. The photographs that are attached as Exhibits B and C hereto were taken in my laboratories and under my supervision contemporaneously with the experiments from which they resulted. When I use the term "we" in this Declaration, I refer to those working under my supervision in my laboratories and to me.

THE FIRST SET OF EXPERIMENTS

3. As I reported in the Fallon I Declaration we administered various growth factors and combinations of them into the intracerebroventricals ("ICV") of both unlesioned and lesioned rats (6-OHDA Parkinson's disease model). See paragraphs 31 and 32. In this model, a lesion is induced in the rat by stereotaxic injection of a solution of 6-OHDA (6-hydroxydopamine) into the rostral border of the substantia nigra-ventral tegmental area ("SN-VTA"), substantially as described in my 2000 PNAS paper (Fallon et al., PNAS, 97(26), pp. 14686-91 (2000)) at p. 14686. *See also* U.S. patent application 09/129, 028 ("the '028 application"), pages 45-46 and 59-61.

4. We administered the growth factors or the combinations of them in this first set of experiments using osmotic minipumps and an implanted cannula, substantially as described in my 2000 PNAS paper at pp. 14686-87. *See also* the '028 application, pages 46 and 60.
We typically administered the growth factor into an intracerebroventricle ("ICV") using the implanted cannula over about a two week period (in some experiments a shorter or longer time period was used) at a rate of about 0.5 μl/hour using the minipump and an about 200 μl solution, containing about 100 μg of the growth factor or the combination of growth factors. In the lesioned rat experiments reported in this first set of experiments, we typically administered the

growth factor solution about two weeks after we had induced the 6-OHDA lesion. In other experiments, we administered the growth factor contemporaneously with induction of the lesion.

5. As described below, in the experiments reported in this first set of experiments, at various times after we had completed administration of the growth factor, we observed the lesioned animals to assess whether or not they had any functional or behavioral improvement in the rotational asymmetry that resulted from the 6-OHDA-induced lesion. *See also* my 2000 PNAS paper at p. 14687. We also sacrificed some of the animals and carried out various histo- and immunohisto-chemical analyses of their brain tissue. *See, e.g.,* the '028 application, pages 46-48 and 61-63 and my 2000 PNAS paper at p. 14687.

6. In the experiments reported in this first set of experiments, we used the following growth factors: EGF, TGF-α, bFGF (also known as FGF-2), aFGF (also known as FGF-1), GDNF, NGF, NT2 and NT3, as well as combinations of TGF-α and FGF; EGF and FGF; and GDNF, TGF-β, NT3 and NT4, in both lesioned and unlesioned animals.

7. I have attached my hand-drawings showing the induced lesions and the ICV administration of the growth factors and combinations that we used in this first set of experiments and the observed results as Exhibit A, Tabs (i), (ii) and (iii).

8. The three Tabs of Exhibit A depict eleven (11) sets of experiments. Each set employed a given growth factor or combination of them (indicated on the far left of the drawing for each growth factor set), ICV administration, and an unlesioned and lesioned animal. In each growth factor set, I depicted one side of the coronal section of the rat mid-brain of the unlesioned animal on the left-hand side of the drawing and of the lesioned animal on the right-hand side of the drawing. In all of my hand-drawings on both the left and right sides of each growth factor set, I used a substantially vertical red line to indicate the location of the implanted cannula and

the ICV administration of the growth factor.[1] In the first set of experiments depicted in each Tab of Exhibit A, I labelled this line "ICV". On the right-hand side of the drawings for each growth factor set (the lesioned animals), I depicted in red the location of the delivery of the 6-OHDA. I also depicted the location and size of the induced lesion as shown by tyrosine hydroxylase ("TH") staining (an immunohistochemical staining protocol for observing the loss of dopaminergic neurons in tissue). *See, e.g.*, the '028 patent application, p. 46. I noted the TH staining in the first set of growth factor experiments in Tab (i) and Tab (iii).

9. We sacrificed some of the lesioned animals, treated as described in paras. 3-6 above, and prepared samples for histological analysis to determine whether any sustained cell proliferation and/or directed migration had occurred. We used several different analyses. *See, e.g.*, the '028 patent application, p. 46-48 and 61-63, and my 2000 PNAS paper at p. 14687. In Exhibit B, I have attached four photographs of one of those analyses -- nestin staining (using monoclonal antibodies against nestin) -- of the samples from three of the lesioned animals described above, i.e., animals treated with two growth factors and one combination of them: TGF-α, EGF and TGF-α/FGF. See labels in upper left hand corner of each photograph in Exhibit B. I have also included a fourth photograph of a nestin-stained sample from a lesioned animal which we treated in much the same way as the lesioned animals described above, with a combination of NT3 and FGF using ICV administration. Nestin is a cytoskeletal marker for early lineage neuronal progenitor cells. Thus, it is a good indicator of neuronal cell proliferation in our experiments. *See* the '028 application, page 71 and Fig 3(b) of my 2000 PNAS paper at page 14689.

[1] In Tab (i) in the aFGF set, I noted that the implanted cannula had nicked ("nick") the ventricle.

10. As can be seen from the four (4) photographs in Exhibit B, neither of the individual growth factors, TGF-α or EGF, and neither of the combinations of growth factors NT3/FGF or FGF/TGF-α, when administered to the lateral ventricles (ICV) of the 6-OHDA lesioned animals, induced any meaningful cell proliferation. TGF-α appears to be the best. But, it too results in no significant cell proliferation. I estimated a few tens of cells. In other experiments, we observed the same minimal cellular response after ICV administration of saline alone. I believe that the minimal proliferation that we observed in these experiments may, indeed, be a response to the injury occasioned by the insertion of the cannula itself.

11. As a result of these nestin-staining experiments as well as other histological experiments on the animals in this first set of experiments, at the bottom of each of Tabs (i), (ii) and (iii) of Exhibit A, I noted the results of the growth factor-ICV experiments for both the unlesioned and lesioned animals:

(i)	no lesion:	"no signif prolif"
	with lesion:	"no signif prolif"
(ii)	no lesion:	"no effects on prolif"
	with lesion:	"lesion ant to SN-VTA/MFB same non effect"
(iii)	no lesion:	"no signif prolif"
	with lesion:	"no signif prolif"

12. In some of the animals, both lesioned and unlesioned, we also carried out behavioral testing using the apomorphine-induced rotation technique, substantially as described in my 2000 PNAS paper at p. 14687. In no case did we observe any behavioral improvement in any of the lesioned animals after the ICV administration of any of the growth factors or the combinations thereof that characterize the various experiments in this first set of experiments. These behavioral experiments demonstrated that the few tens of cells that we observed in some

of the animals were not meaningful or significant in terms of ameliorating the effects of the lesion.

13. Therefore, this first set of experiments demonstrated that infusions of various growth factors and various combinations of them into the ICV led to no significant, meaningful, or useful cell proliferation or migration in either unlesioned or lesioned animals, and had no effect on behavioral or functional recovery in lesioned rats (6-OHDA model).

THE SECOND SET OF EXPERIMENTS

14. As I reported in the Fallon I Declaration, intrastriatal administration of TGF-α in lesioned animals (6-OHDA model) leads to a sustained, massive cellular proliferation and mass migration of cells. *See*, *e.g.*, para. 12 and Figure 1. *See also* Figures 1-3 of my 2000 PNAS paper, pages 14688-89. By contrast, I reported in the Fallon I Declaration that EGF administration to the striatum of lesioned rats did not produce that effect. *See*, *e.g.*, para. 16. *See also* the '028 application, p. 63.

15. I now provide actual experimental data underlying these statements, as well as data from similar experiments where we used other growth factors. We carried out these experiments substantially as described in my 2000 PNAS paper, pp. 14686-87 and in the '028 application, e.g. pages 45-46 and 59-61. We used five different growth factors: TGF-α, EGF, aFGF (FGF-1), bFGF (FGF-2) and NGF. We administered each factor intrastriatally into a 6-OHDA-lesioned rat either contemporaneously with, or subsequent to, induction of the lesion.

16. For each of these experiments, we carried out a series of morphological and histochemistry analyses. I have attached, as Exhibit C hereto, photographs of the results of one of these sets of experiments – staining for nestin – after intrastriatal administration of EGF, bFGF (FGF-2), NGF and aFGF (FGF-1) to lesioned rats (6-OHDA model).

17. In Exhibit C, there are four panels, labelled A-D. They reflect the results of the intrastriatal administration of each of the following growth factors to the lesioned animals:

a. EGF

b. bFGF (FGF-2)

c. NGF

d. aFGF (FGF-1)

18. These photographs show convincingly that there is at best very minimal cell proliferation – in the order of tens of cells – after intrastriatal administration of EGF, bFGF, NGF and aFGF to the lesioned animals. We observed a similar response when saline alone was administered intrastriatally. Again, this suggests that the minimal response is due to the injury occasioned by the insertion of the cannula.

19. The results of a similar experiment with TGF-α are shown in Figure 3(b) of my 2000 PNAS paper. *See* p. 14689. They are also shown in Exhibit D attached hereto, a hand-drawing that I made in my notebook contemporaneously with this TGF-α experiment. The hand-drawing and Figure 3(b) of my 2000 PNAS paper show the nestin-positive cells after TGF-α infusion to the striatum of a 6-OHDA lesioned rat. Both demonstrate a massive proliferation of neuroprogenitor cells and their migration to form a prominent striatal ridge. For example, my hand-drawing (Exhibit D) shows the site of TGF-α injection and the ridge of cells (the dark cross-hatched region) that had formed and migrated away from the site of administration. The arrows in Figure 3(b) of my 2000 PNAS paper show a similar ridge of proliferated and migrated cells induced by intrastriatal administration of TGF-α to lesioned rats (6-OHDA model). See also Figure 2 of my 2000 PNAS paper.

20. Figure 3 of my 2000 PNAS paper (p. 14689) shows other characteristics of the cellular proliferation and migration induced by intrastriatal administration of TGF-α to lesioned

rats (6-OHDA model), e.g., silver staining (Figure 3(c) suggesting outward migration of the cells; BrdUrd staining (Figure 3(d), indicating the cells have recently been generated de novo); and tubulin staining (Figure 3(e), indicating the cells were of neuronal lineage). Figure 2 of my 2000 PNAS paper likewise shows the time course of the cell proliferation, ridge formation, and migration of the striatal ridge of cells.

21. In some experiments in which we administered TGF-α intrastriatally to lesioned rats (6-OHDA model), I estimated the number of new neuronal progenitor cells that had been induced by TGF-α and had formed and were located in the striatal ridge. *See*, *e.g.*, my hand drawings and "calculations" in Exhibit E, Tabs (i) and (ii). In Tab (i), I estimated the number of progenitor cells in the 20+ cell-wide ridge to be about 1.5 million cells. In Tab (ii), I estimated the number of cells in the 4 mm long, 0.5 mm wide striatal ridge to be 4 million cells, and indicated that 1 million cells was a "conservative guess." Both of these estimates demonstrate that intrastriatal administration of TGF-α to the lesioned rats induced cellular proliferation at more than 4-orders of magnitude (10,000-fold) over the number of cells induced by ICV administration of TGF-α and the many other growth factors (indeed by saline alone) that I used for my experiments to both lesioned and unlesioned rats (see the first set of experiments) and by striatal administration of EGF, NGF, aFGF and bFGF to the lesioned rats (see the second set of experiments).

22. We drew the following conclusions from this second set of experiments. *Only* administration of TGF-α to the striatum of animals, either contemporaneously with, or subsequent to, a 6-OHDA-induced lesion, led to massive neuronal cell proliferation and mass migration of the cells to form a striatal ridge of cells. By contrast, striatal administration of other growth factors (aFGF, bFGF, EGF and NGF) did not result in such cell proliferation or ridge

formation (Exhibit C). Moreover, the TGF-α induced cell proliferation results (Exhibit D and Figure 3(b) of my 2000 PNAS paper) differed by many orders magnitude as compared to that induced by the other growth factors (which proliferation was about the same as we observed using saline alone).

23. We also conducted a series of experiments to determine whether or not there were any functional and behavioral effects of the massive cell proliferation and migration that we observed in the 6-OHDA lesioned rats treated intrastriatally with TGF-α. These experiments are described in detail in my 2000 PNAS paper (*see* p. 14690). As compared to a cerebrospinal fluid (aCSF) control, the lesioned rats treated with TGF-α showed a statistically significant improvement in rotational behavior even when treated 14 days after the lesions were induced. Other experiments demonstrated that when we administered the TGF-α contemporaneously with the 6-OHDA injection (to induce the lesion) the animals were protected from the normally-observed rotational behavioral asymmetry over a 4 week period. *See* my 2000 PNAS paper at p. 14690.

24. From these behavioral and functional experiments and observations, we concluded that the massive cell proliferation and ridge formation that result when TGF-α is administered intrastriatally to 6-OHDA-lesioned rats has an easily observed effect on behavior and function in the animal both when used well after the lesion is induced and when used contemporaneously with induction of the lesion.

25. I hereby further declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine, imprisonment, or both, under Section 1001 of Title 18 of the

United States Code and that such willful false statements may jeopardize the validity of any patent issuing from this application.

Dated 20/10/2010

James H Fallon, Ph.D.

EXHIBIT A

(i)

REDACTED

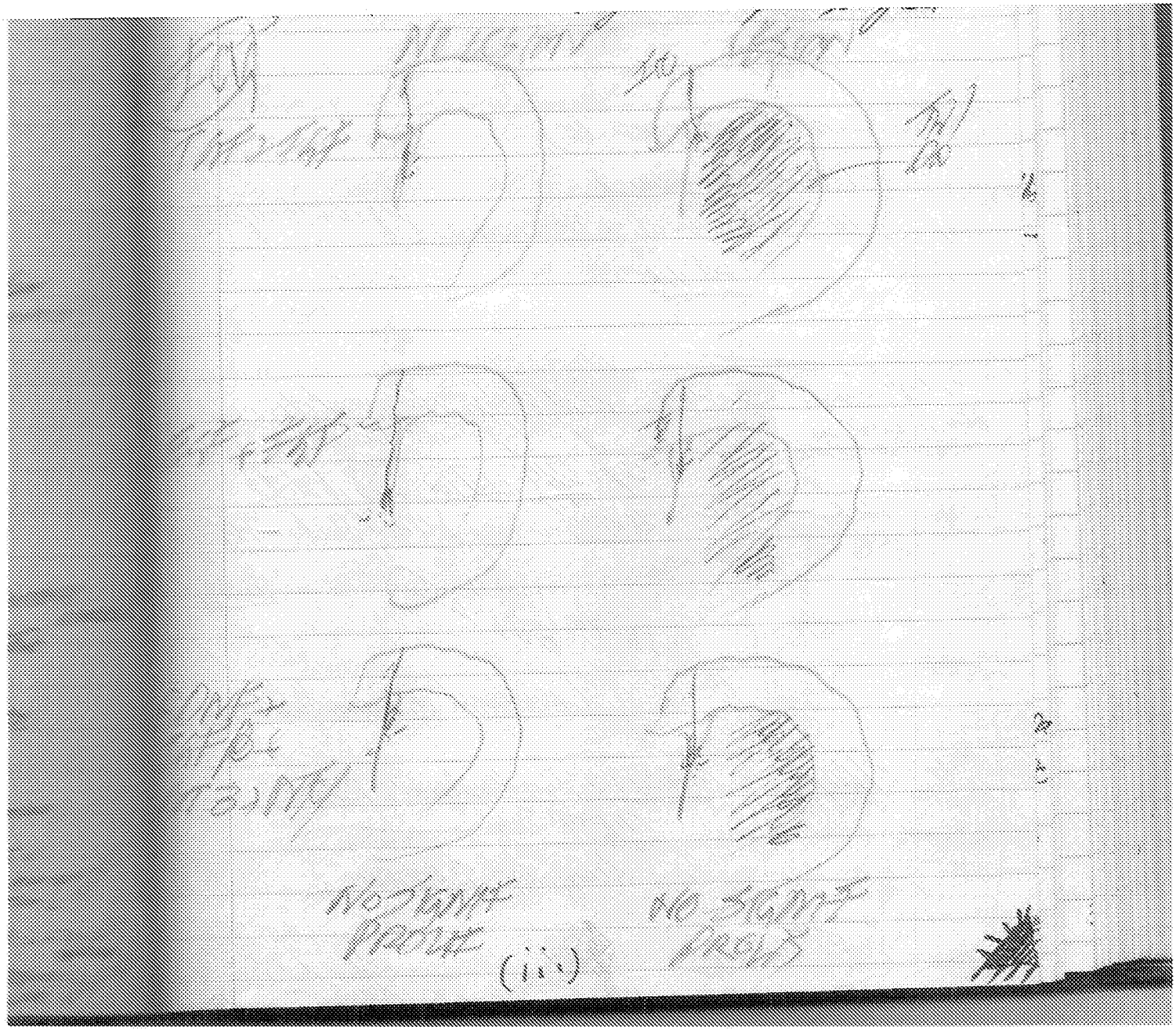


EXHIBIT B


ICV TGFa


ICV NT-3 FGF


ICV EGF

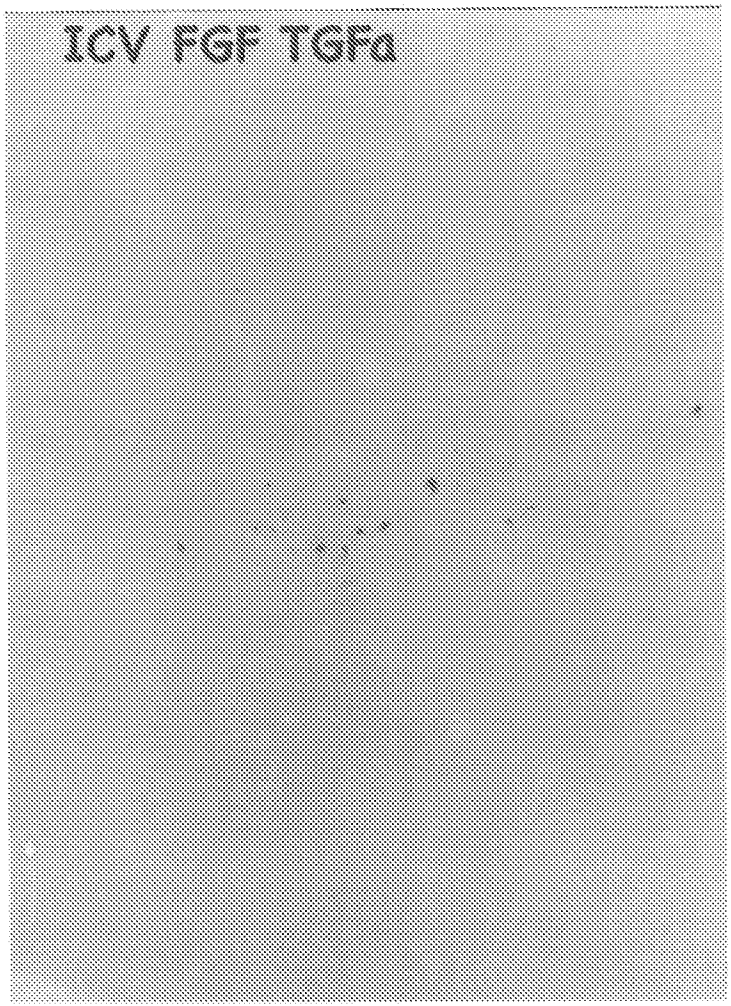

ICV FGF TGFa

EXHIBIT C

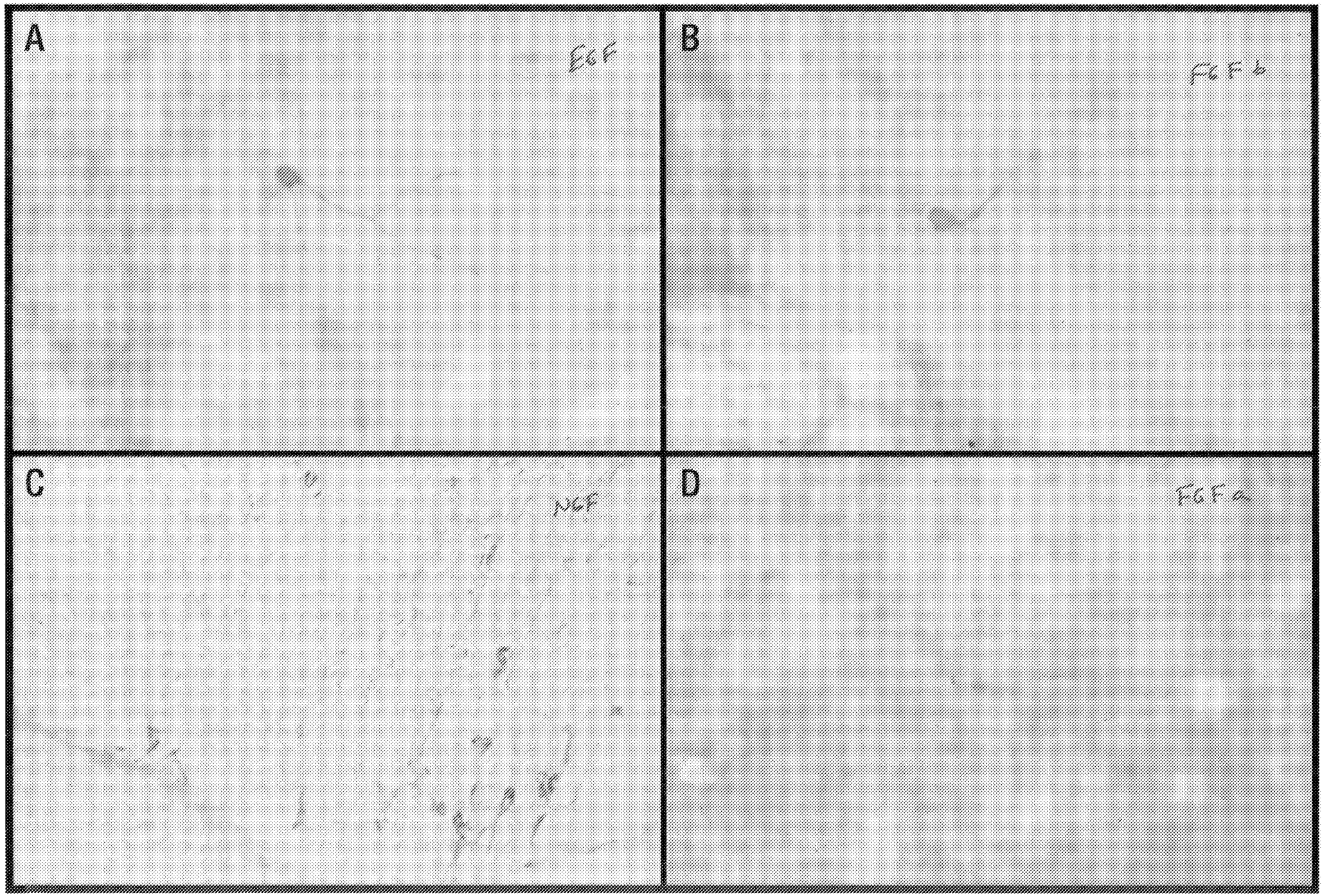

A
EGF
B
FGF b
C
NGF
D
FGF a

EXHIBIT D

Remarks

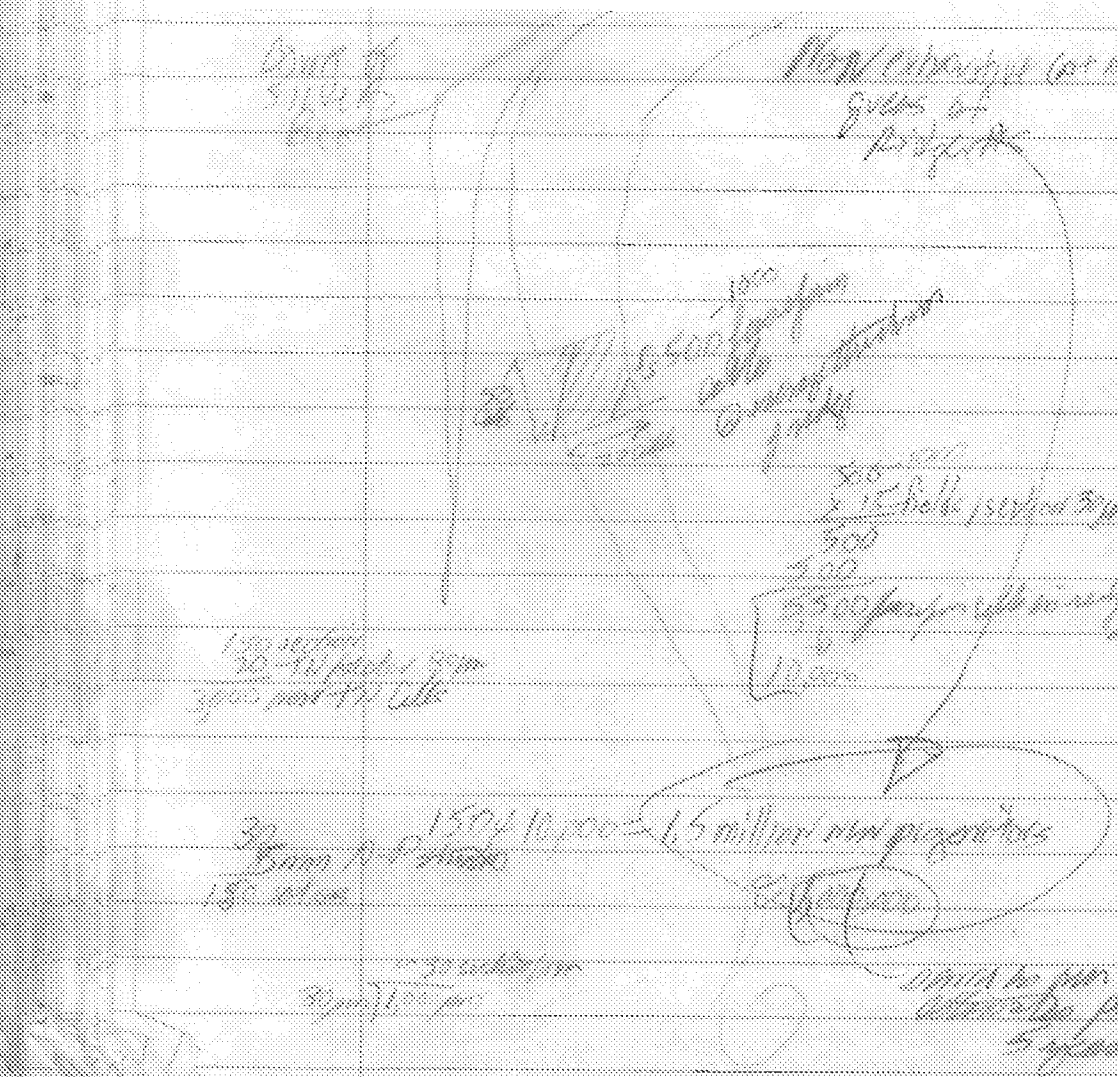
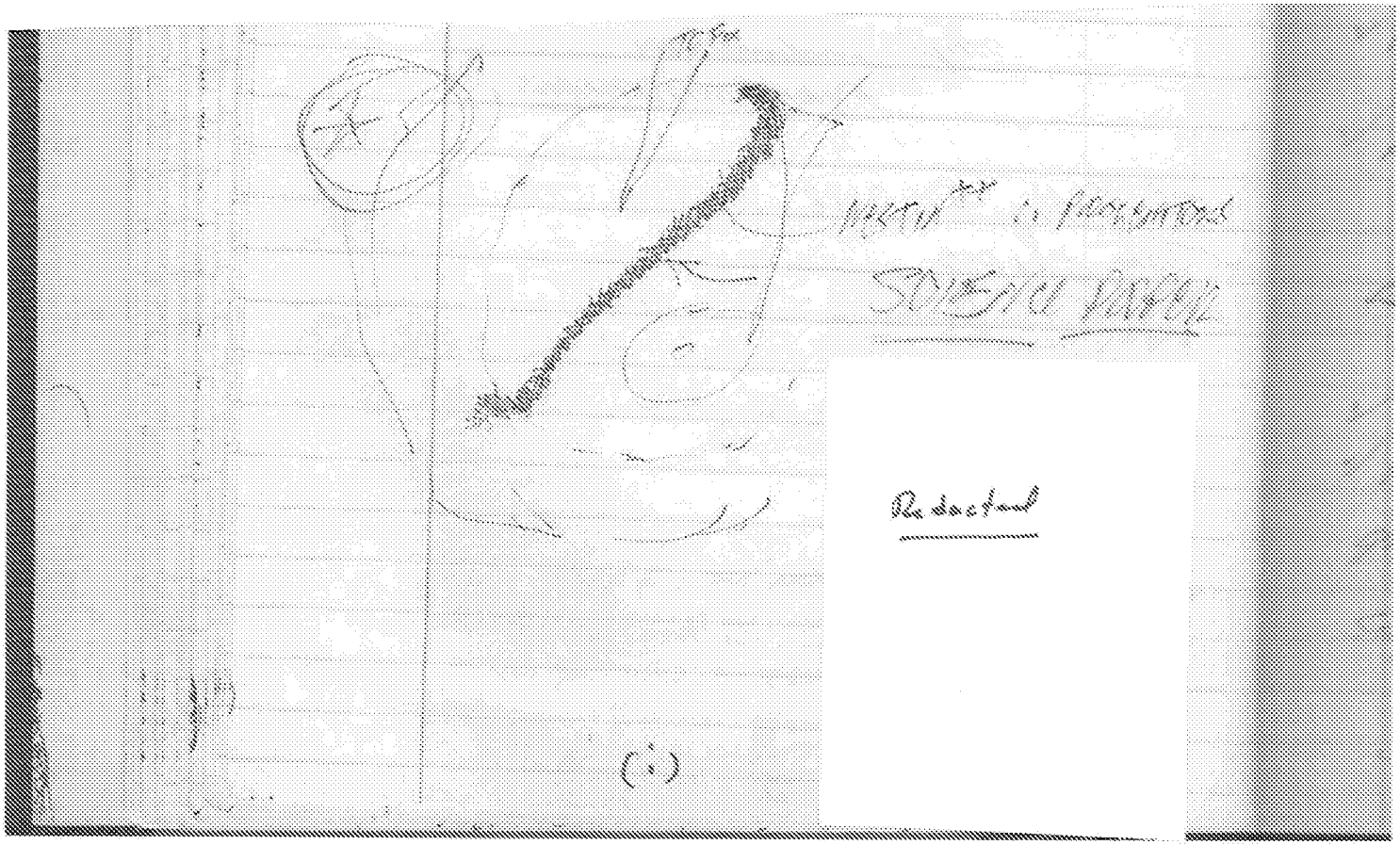


EXHIBIT E

REDACTED



REDACTED

RADIO OCC ESTIMATION

20 mins (5 cm × 500 cells/ml = 1 per 0.2 ml load) x 4 mm long
10 mm × 4 mm × 1 mm deep

1:3 → 5.2 (20) = 8 mm long

2 (105–350 cells/mm²) = 3 mm

3 mm wide M-L

5 mm D-V

Length of probe 24 mm

10 x 5 mm wide

Small Cerebellar Area

Septum

(ii)